Comparative features of hepatitis C virus infection in humans and chimpanzees

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Introduction

The common chimpanzee *Pan troglodytes* is the only species other than man with known susceptibility to persistent, probably life-long hepatitis C virus (HCV) infection. This animal model provided at least three important insights into the nature of post-transfusion hepatitis (PTH) not caused by hepatitis viruses A or B. First, transmission of disease to chimpanzees by serum or plasma from individuals with acute and chronic non-A, non-B PTH firmly established the involvement of a blood-borne agent with the ability to persist in its human host [4, 33, 63, 82, 83]. Subsequent studies suggested that serum samples from chronically infected subjects usually contained low titers of the agent, ranging from less than 10^2 to 10^3 chimpanzee infectious doses (CID) per ml [13, 29, 84]. Second, plasma from subjects with PTH was still infectious for chimpanzees after passage through an 80-nm pore-size filter or treatment with chloroform (reviewed in [13, 63, 66]), and thus it was established with reasonable certainty that the responsible agent was a small, enveloped virus. Third, chimpanzees were central to the identification of HCV as the agent of non-A, non-B hepatitis. Standard virologic assays failed to identify a virus in the tissues of infected individuals. Indeed, cell culture systems supporting robust replication of HCV are still not available and virus particles have only recently been identified in hepatocytes of an acutely infected chimpanzee [79].

Serologic approaches for identifying viral antigens associated with non-A, non-B hepatitis were probably hampered, at least in part, by low levels of antiviral antibodies and HCV proteins in serum or liver tissue. A high-titered virus stock developed by serial passage of contaminated factor VIII concentrate through two chimpanzees finally facilitated identification of HCV by molecular methods. Plasma pooled from the second recipient animal (CDC no.910) at times of peak alanine aminotransferase (ALT) activity contained greater than 10^6 CID per ml serum [13]; reverse transcription of pelleted nucleic acids with random primers and cloning into λgt11 cDNA

bacterial expression libraries led to the identification of a single molecular clone des-
ignated 5-1-1 that encoded a polyprotein recognized by serum antibodies from humans
presumed to have non-A, non-B hepatitis [19]. This clone specifically hybridized to
RNA extracted from tissues of infected but not of uninfected chimpanzees and hu-
mans. Moreover, it expressed a fusion protein from the NS3 and NS4 regions that was
recognized by a coded panel of sera from patients with documented non-A, non-B
PTH [19]. From this starting point, the positive-strand RNA genome of the first virus
isolate, designated HCV-1, was rapidly cloned and sequenced.

Chimpanzees remain the only reliable animal model for studying the pathogenesis
of human HCV infection. One key question is whether the disease and virus-specific
immune responses are equivalent in these two closely related species. This chapter
compares the salient features of infection in humans and chimpanzees, including liver
histopathology, the natural history of infection, patterns of HCV replication, and the
nature of humoral and cellular immune responses.

Liver histopathology and natural history of HCV infection

Acute HCV infection of chimpanzees is usually marked by necroinflammatory lesions
in the liver, an increase in serum transaminase values, and ultrastructural alterations
in hepatocytes [4, 33, 34, 60, 64, 76, 82, 83]. Focal degeneration of single or small
groups of hepatocytes into acidophilic bodies, bile duct alterations, and portal tract
inflammation that includes formation of lymphoid aggregates are usually evident [11,
62]. Hepatocellular injury and inflammation are often obvious about 1 to 2 weeks prior
to the first major elevation of transaminase activity [62]. These histologic features are
remarkably consistent with those of acute hepatitis C in humans as reviewed by
Scheuer et al. in chapter 3 of this volume. Examination of chimpanzee hepatocytes
by electron microscopy also revealed ultrastructural changes that appear as 150- to
300-nm double-walled membranous tubules in the cytoplasm [34, 60, 76] probably
caused by proliferation of smooth endoplasmic reticulum.

Chronic hepatitis C is considered less severe in chimpanzees than humans. Ultra-
structural and transaminase abnormalities can disappear in infected animals only to
recur 1–2 years later [11]. In two representative studies, chimpanzee liver sections
examined by light microscopy had increased numbers of sinusoidal cells, some fo-
cal necrosis, and irregular portal inflammation [11, 62]. Lymphocytes were generally
sparse in the lobular parenchyma. These histologic features are similar to those in
human chronic persistent hepatitis (CPH), a disease process that has been considered
benign and self limited. Liver inflammation in persistently infected humans can also
be mild [3, 23] or perhaps even absent in some rare cases [14]. Significantly, how-
ever, serious pathology involving interface inflammation and erosion of limiting plate
hepatocytes around portal tracts is observed in humans but not chimpanzees. This
chronic active hepatitis (CAH) is generally considered to have a worse prognostic
outcome, and could represent an important but as yet unexplained difference in the
evolution of disease between the two species.

Natural history studies support the contention that liver pathology progressively
worsens in some humans but rarely if ever in chimpanzees. Deterioration of liver
architecture has been confirmed by serial biopsy of human subjects, and progression
from CPH to CAH, cirrhosis, and even hepatocellular carcinoma (HCC) over a period
of several decades has been documented [75]. The time course for individual subjects

is highly variable, however, and irreversible liver disease sometimes develops after less than 5 years of infection [75]. Evidence that chimpanzees follow a similar progressive disease course is still lacking. Of note, however, is the development of HCC in a chimpanzee infected about 7 years earlier with HCV [55]. This is a rare event in animals not normally exposed to hepatocellular co-carcinogens, but it remains to be seen if symptomatic liver disease will eventually develop in more chimpanzees infected over the past one to two decades.

Virologic features of HCV infection in humans and chimpanzees

Chimpanzees have been challenged with a variety of infectious inocula from individuals with non-A, non-B hepatitis, including serum, plasma, and liver homogenates collected during acute or chronic phases of disease, peripheral blood mononuclear cells, and contaminated blood products such as factor VIII concentrate [13]. Most of these experiments preceded the discovery of HCV, and thus the titer and genotype of challenge virus were undefined. Exceptions are those animals infected by two well-characterized challenge stocks. In addition to plasma from chimpanzee CDC no.910 that was the source of the genotype 1a HCV-1 molecular clone [13], acute-phase plasma from a human subject (designated H) who developed chronic non-A, non-B PTH has been studied extensively. This plasma, which was the source of the HCV-H77 (genotype 1a) genomic consensus sequence [57], has a CID titer of about $10^{6.5}$/ml [29]. This is in good agreement with results from the branched DNA probe assay, which demonstrated that the ratio of HCV RNA genome equivalents to CID was about 1:1 in H serum [5]. Interestingly, chronic-phase plasma from a second subject designated F had a CID of $< 10^2$/ml [29] and a genome equivalent:CID ratio of greater than 10 000:1 [5]. Reasons for these differences are not clear, but could reflect a high level of defective genomes or immune complexes in the plasma of this subject.

Even though relatively few animals have been infected to date, some common features of HCV replication in chimpanzees and humans can be identified. First and foremost, the frequency of persistent infection is at least 70% for humans and is probably similar for chimpanzees [1, 9, 13]. Precise estimates are lacking due to the small numbers of animals used in most studies, and as noted above challenge stocks were usually not well characterized. Thus possible influences of HCV dose or genetic complexity on the outcome of infection are as yet poorly understood.

Second, virus replication during acute infection is an early event that precedes the onset of liver disease by several weeks. Replication of HCV in hepatocytes as early as 3 days after experimental infection of a chimpanzee was demonstrated by in situ hybridization (ISH) [56], and virus particles have recently been visualized in hepatocytes from an acutely infected animal [79]. Viremia is detectable by reverse-transcriptase (RT)-polymerase chain reaction (PCR) amplification of the HCV RNA genome, with positive signals detected in the plasma of some animals within 3–4 days of infection [9, 77]. In most studies HCV is typically present at the first sampling point after infection (usually at 7–10 days) [1, 9, 26, 80], but for a minority of animals several weeks can elapse before virus sequences are first detected [1, 31]. Despite HCV viremia early in the acute phase of infection, biochemical markers of liver damage are generally not elevated until about 7–10 weeks post-infection [1, 9, 26, 77]. Preliminary studies demonstrating HCV RNA in hepatocytes by ISH long before peak

ALT activity or the appearance of ultrastructural changes are consistent with these findings [2]. Similar observations have been made in humans with PTH where the time of infection can be accurately pin-pointed; virus is frequently detectable within 1 week of infection [24], and recent studies with branched DNA molecular probes revealed that virus titers can exceed $10^{7.5}$ genome equivalents/ml serum several weeks before biochemical evidence of liver damage [5]. Immunohistochemical detection of viral antigens in a high percentage of hepatocytes during acute phase in chimpanzees [44] and humans [71] is consistent with a burst of virus replication immediately after infection.

Third, patterns of plasma viremia are generally similar in humans and chimpanzees with chronic hepatitis C. There can be a transient decline in serum viremia at around the time of initial transaminase elevations [5, 24]. Mechanisms that might contribute to reduced virus replication during this period of time are not well defined, but it has been suggested that there can be a brief latent period when host defenses are at least partially effective [2]. This finding is not universal, however, and highlights the variable course of virus replication in individual humans or chimpanzees. Longitudinal studies of chronically infected humans indicate that viremia is consistently detectable over a period of years to decades regardless of the severity of liver disease [24]. Quantities of viral RNA in the serum of chronically infected humans are highly variable from about 10^2 to 10^8 genome equivalents/ml. Virus titers in chimpanzees can also vary over a 4–6 log range, and at least in some animals viremia can be intermittent for periods of several months [1, 9]. Finally, it has been demonstrated that HCV circulates as a quasispecies in chronically infected chimpanzees and humans. HCV mutation rates in both species appear to be remarkably similar, at around 1.44– 1.92×10^{-3} base substitutions per genome site per year [57, 58].

Humoral immune responses

Seroconversion to HCV antigens has been documented retrospectively using serial plasma samples collected from infected chimpanzees Antibodies from most animals recognized C-100, a recombinant fusion protein representing the C terminus of NS3 and most of NS4 contained in first generation immunoassays [1, 12, 26, 31, 48, 73, 77]. Several weeks to months elapsed between the time of infection and seroconversion against this antigen, mirroring observations in human hepatitis C [1, 12, 26, 31, 73, 77]. Inclusion of additional antigens from the nucleocapsid and non-structural regions shortened this window considerably in humans and chimpanzees; although highly variable, the time to seroconversion in both species averaged about 15–20 weeks [1, 9, 26, 31]. The timing of seroconversion or pattern of responsiveness to individual HCV antigens does not appear to predict resolution versus persistence of infection, although as noted below relatively little is known about differences in the functional activity of antiviral antibodies.

Persistent infection is established in chimpanzees and humans despite the presence of antibodies against most viral proteins [1, 12, 26, 31, 48, 73, 77], including the envelope glycoproteins E1 and E2 [17, 20]. Virus isolate-specific neutralizing antibodies have been described in the serum of chronically infected subject H; antibodies collected at different points over a 12-year period varied in their ability to neutralize infectivity of autologous virus isolates for chimpanzees [27] or human cell lines [78]. Two lines of evidence suggest that these neutralizing antibodies target envelope anti-

gens. First, chimpanzees immunized with recombinant envelope glycoproteins were protected against intravenous challenge with a low dose of the homologous virus isolate [20]. Second, specific antibodies can block in vitro binding of recombinant E2 protein or HCV virions to cells [70, 93]. Epitopes involved in neutralization are not fully defined, but considerable attention is focused on hypervariable region 1 (HVR-1) located near the N terminus of E2. This 34-amino acid domain spanning amino acids 384–414 of the HCV polyprotein is the most variable in the entire genome and genetic diversification is observed during the course of persistent infection in humans and chimpanzees (reviewed in [15]). Indirect evidence suggests that in both species, the HVR-1 domain contains linear epitope(s) targeted by neutralizing antibodies that exert selection pressure. For instance, anti-HVR-1 antibodies from human subjects sometimes fail to recognize variants that emerge as the dominant master species later in infection [36, 72, 85, 89]. Mutations in this domain in experimentally infected chimpanzees have been linked to the development of specific antibodies [87], and in one chronically infected human subject with hypogammaglobunemia there was minimal drift in the HVR-1 sequence over a 30-month period [46]. HVR-1 sequences are also more conserved in virions associated with immune complexes. Highly variable sequences in free virions, which are thought to be more infectious, could result from a process of immune selection [18]. Direct evidence now indicates that anti-HVR-1 antibodies block infectivity of HCV; a hyperimmune serum from rabbits immunized with an HVR-1 peptide neutralized the infectivity of a homologous HCV isolate for chimpanzees [28]. Notably, quasispecies variants with heterologous HVR-1 sequences had residual infectivity [28].

One difference in the serologic response may be a reduced frequency of seroconversion against structural proteins in chimpanzees versus humans [1, 20, 31, 48, 52, 88]. Mechanism(s) underlying restricted antibody responses against the core, E1 and E2 proteins in chimpanzees are unclear, but could reflect suppression or elimination of antigen-specific B cell clones. B cells infected or sensitized with viral antigens via cell-surface immunoglobulin are targeted for elimination by antigen-specific cytotoxic T lymphocytes (CTL) [7, 61]. In this situation, one could speculate that species differences in the susceptibility of B cells to productive HCV infection, the kinetics or strength of CTL directed against structural antigens, or genetic restriction of CTL activity could influence patterns of antibody responses.

Cellular immune responses

The observation that HCV replicates for decades in chimpanzees and humans, and that both species are susceptible to reinfection or superinfection even by homologous strains [25, 47, 59, 65], implies that cellular immune responses are weak. On the other hand, indirect evidence does suggest that host immunity provides at least partial control of virus replication, while at the same time contributing to liver disease. There is no obvious temporal relationship between virus replication and biochemical evidence of liver disease. As reviewed above, virus replicates for several weeks before transaminase levels increase, suggesting involvement of host factors in hepatocellular damage. ALT values and liver histopathology are also normal despite ongoing virus replication in some chronically infected animals. In addition, in one preliminary study levels of plasma viremia were increased in chimpanzees treated with cytotoxic or immune suppressive drugs [45]. At the same time biochemical markers of liver disease

were reduced and, significantly, withdrawal of drugs reversed these trends. Similar observations have been made in chronically infected humans treated with these agents to prevent allograft rejection or to enhance therapeutic effects of interferon-α (IFN-α) (reviewed in [69]). Finally, hepatocytes in both species express cytoplasmic HCV antigens that should be processed for presentation to immune T cells [6, 44, 71]. Major histocompatibility complex (MHC) class I molecules are nearly undetectable on the surface of normal human hepatocytes, but expression is up-regulated in chronic hepatitis C. Indeed, antigen-containing hepatocytes have MHC class I and adhesion molecules required for T cell recognition [6]. Similar mechanisms are probably operative in infected chimpanzees; although direct immunohistochemical evidence of increased MHC class I expression on hepatocytes is still lacking, levels of mRNA encoding MHC class I molecules and IFN-γ are elevated [37].

Interactions between HCV-specific T lymphocytes and productively infected hepatocytes have been difficult to study because a suitable cell culture system for propagating the virus is still lacking. It is still not clear, therefore, if its replication cycle can be interrupted by direct T cell-mediated cytolysis or antiviral lymphokines, and possible effects of infection on antigen-presentation pathways of hepatocytes remain to be defined. Nevertheless, it has been possible to measure responses by $CD8^+$ and $CD4^+$ T lymphocytes using recombinant vaccinia viruses expressing HCV-coding regions and synthetic viral peptides or subunit proteins.

CD4$^+$ T lymphocytes

All studies to date of HCV-specific, MHC class II-restricted $CD4^+$ T lymphocytes have been conducted in humans subjects and are reviewed by Abrignani elsewhere in this volume. Briefly, peripheral blood mononuclear cells (PBMC) from many chronically infected subjects respond to at least one viral antigen in standard lymphoproliferation assays [10, 30, 32, 49–51, 74, 86, 92]. Simultaneous responses to more than two or three HCV proteins are rare, however, and stimulation indices are generally low when compared with those induced by standard recall antigens [10, 30, 32, 50]. There are very limited data on intrahepatic $CD4^+$ T lymphocytes in chronic infection. One study has demonstrated that NS4-specific T cell lines derived from the peripheral blood and liver of an HCV-infected individual were functionally distinct [53], suggesting that the liver microenvironment could influence antiviral activities of the cells. Because access to liver tissue in humans is limited, the chimpanzee model may be ideal for further investigation of these issues.

Strong $CD4^+$ T cell responses may contribute to a favorable outcome of HCV infection. One study suggests that chronically infected subjects with less severe liver disease are more likely to have positive lymphoproliferative responses, possibly against the highly conserved core protein [10]. Two other studies demonstrated a strong correlation between vigorous lymphoproliferative responses during the acute phase of disease and spontaneous resolution of viremia [21, 54]. NS3 appeared to be the dominant target of this response in one study [21], although in the second $CD4^+$ T cells from subjects with resolved disease appeared to be broadly directed against most HCV proteins [54]. Memory responses in these recovered individuals appear to be long lived. One key unanswered question is whether these T lymphocytes contribute directly to control of virus replication, perhaps by production of cytokines that inhibit

virus replication, or by supporting the growth and differentiation of other immune effector cells such as $CD8^+$ T lymphocytes.

CD8+ T lymphocytes

$CD8^+$ CTL that are specific for HCV antigens have been detected in the peripheral blood and liver of chronically infected humans. Koziel et al. [41, 42] were the first to demonstrate intrahepatic virus-specific CTL in chronic HCV infection (reviewed by Koziel and Walker in this volume). Clonal $CD8^+$ cell lines killed autologous B cells infected with vaccinia viruses encoding HCV structural and non-structural antigens, and a number of epitopes presented by HLA-A and B alleles have been identified [41–43]. Besides HCV-specific lytic activity, antigen-specific stimulation of these cells also results in production of cytokines such as interleukin-8 and granulacyte-macrophage-colony-stimulating factor that could have important influences on the inflammatory response in the liver [43].

Similar results have been obtained in two chimpanzees chronically infected with HCV-1 [22, 40]. Intrahepatic $CD8^+$ T cell lines derived from chimpanzees Ross (CH-503) and Blair (CH-458) during the acute phase of disease killed autologous transformed B cell lines expressing the structural (core, E1, and E2) and some non-structural (NS2 and NS3) proteins of this genotype 1a strain [22]. Synthetic peptides were used to define minimum optimal epitopes of about eight to ten amino acids recognized by seven CTL lines in the context of class I molecules encoded by the *Pan troglodytes* (Patr) histocompatibility complex [40, 90]. It is noteworthy that alleles of human and chimpanzee MHC class I A, B, and C loci have a high degree of sequence homology, but reagents for identifying individual human class I molecules are not adequate for chimpanzee studies. Indeed, relatively few chimpanzee class I molecules were characterized until recently and rapid genetic or serologic assays for typing are not yet available. To facilitate identification of histocompatibility molecules presenting HCV peptides, all Patr class I genes from both animals were cloned and transfected into a MHC class I-deficient cell line. The pattern of lysis against these cell lines sensitized with the seven epitopic HCV-1 peptides was then defined; results of these studies confirmed that the chimpanzee $CD8^+$ T cells were MHC class I restricted, with Patr A and B molecules presenting two and five epitopes, respectively [40].

A key unanswered question is how the virus manages to evade control by these intrahepatic CTL. Selection of viruses that are not recognized by $CD8^+$ CTL because of mutations in MHC class I-restricted epitopes is one possibility. It is noteworthy that few of the seven epitopes recognized by cells from chimpanzees Ross and Blair were conserved when compared with published sequences of other HCV genotypes [40]. Synthetic peptides representing these genotypic variants in most cases failed to sensitize target cells for lysis by the effector cells specific for HCV-1 genotype 1a sequences [40]. One notable exception was an epitope spanning amino acids 1445–1454 of NS3 recognized by a $CD8^+$ T cell line from chimpanzee Ross. This sequence was conserved in all genotypes, and multiple molecular clones generated from the HCV-1 quasispecies used to infect the animal were identical with the mastercopy [90]. Given this high degree of conservation, it was hypothesized that this sequence might be functionally constrained from mutating to escape the T cell response. Remarkably, however, complete replacement of the quasispecies mastercopy was observed at about

3–4 months post-infection [90]. A conservative amino acid mutation at position 4 of the epitope blocked recognition by four independently derived T cell lines, and new T cell populations directed against the variant were not detected in follow-up studies.

Escape mutation is likely to be only one of many mechanisms by which HCV evades immune recognition. Limited numbers of humans and chimpanzees have been studied to date, but it appears that in many cases $CD8^+$ T cells recognizing HCV antigens are present at low or undetectable levels in persistent infection ([43] and C.M. Walker, unpublished). Mechanisms contributing to this apparently weak response in most individuals are not understood. Serial liver biopsy specimens can be obtained from chimpanzees, and thus this animal model may be ideal for defining the breadth and evolution of intrahepatic $CD8^+$ CTL activity in acute and chronic HCV infection.

HCV-specific $CD8^+$ CTL have also been detected in the peripheral blood of chronically infected humans [8, 16, 35, 38, 39, 67, 68, 81, 91]. As reviewed in an accompanying chapter by Chang et al., most of these studies were facilitated by knowledge of the amino acid motifs that govern binding of antigenic peptides to HLA class I molecules. Peptides of nine to ten residues in length with a leucine or methionine at position 2 and a hydrophobic amino acid with an aliphatic side chain in the last position are predicted to bind to the HLA-A2.1 molecule. Scanning the HCV polyprotein for peptides that fit this motif has led to the identification of peripheral blood CTL directed against structural and non-structural proteins of the virus [8, 16]. CTL percursor (CTLp) frequencies against these HLA-A2.1-restricted HCV epitopes have been defined in chronically infected volunteers [67, 68]. Interestingly, frequencies for individual CTL populations were generally lower than those against HLA-A2.1-restricted epitopes of influenza, and in most cases were not correlated with virus load or flares of liver disease [67]. On the other hand, an inverse relationship between the combined CTL response against all defined HLA-A2.1-restricted epitopes of HCV and virus load has been noted [68]. Quantification of CTLp frequencies during the acute phase of disease in resolved versus persistent infections could reveal additional insights into factors that influence the outcome of infection. The chimpanzee model may be relevant for these studies, and identification of peptide binding motifs for recently described Patr MHC class I molecules cloned from chronically infected animals may be an important first step in that direction.

Epitope prediction is obviously limited to those MHC class I alleles with known peptide binding motifs and, thus, the repertoire of responding T cells is only partially defined by this approach. An alternative is to use pools of overlapping synthetic peptides spanning different HCV proteins to stimulate virus-specific CTL from peripheral blood. Kita et al. [38, 39] successfully used this strategy to identify HCV core-specific epitopes recognized by HLA-B44- and B60-restricted CTL in the peripheral blood of chronically infected subjects. Expansion of these CTL populations from peripheral blood required several rounds of peptide stimulation, presumably because of low precursor frequencies.

We recently used a similar approach to follow the evolution of HCV core-specific CTL responses in the peripheral blood of chimpanzees experimentally infected with the virus. Peptides of 20 amino acids in length offset by 10 residues were synthesized. Nineteen peptides spanning amino acids 1–200 of the HCV-1 polyprotein were organized into three pools; pools 1 and 2 contained six peptides each, spanning amino acids 1–70 and 61–130, respectively. Pool 3 contained seven peptides representing amino acids 121–200. PBMC collected from the two chimpanzees at the indicated times after infection were cultured with the peptide pools for 10 days, when $CD8^+$ T
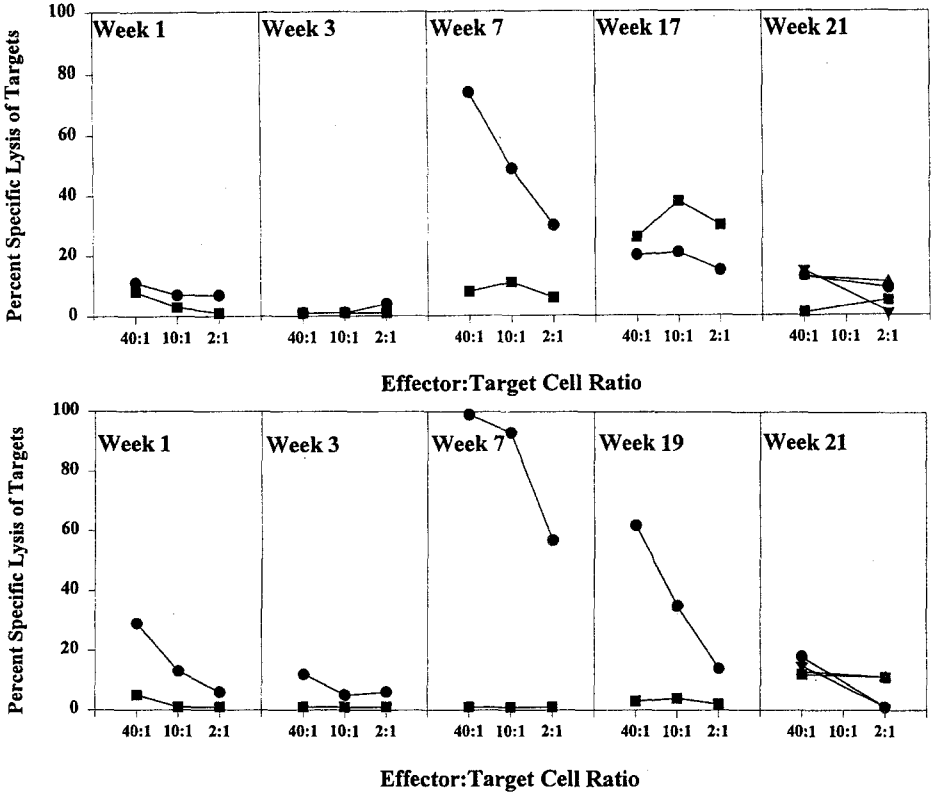
Figs. 1,2 Peripheral blood mononuclear cells recovered from two chimpanzees at the indicated times after hepatitis C virus type 1 (HCV-1) infection were cultured for 10 days in interleukin-2-containing medium with synthetic peptides representing amino acids 121–200 of the HCV-1 core. CD8$^+$ T cells were then enriched using magnetic beads coupled to anti-CD8 monoclonal antibodies. These effectors from chimpanzees Talbot (CH-561, Fig. 1) and Tardy (CH-445, Fig. 2) were tested for lytic activity against autologous B cell targets that were untreated (▼) or sensitized with homologous pool 3 peptides (●) or an irrelevant peptide pool representing another region of core (■). Data are shown for three effector:target ratios after a 4-h assay

cells were enriched by immunoaffinity techniques and tested for lytic activity. Pools 1 and 2 representing the first 130 amino acids of core failed to stimulate HCV-specific CTL activity from the peripheral blood of either chimpanzee at any of the time points tested (data not shown). Pool 3, representing amino acids 121–200, stimulated strong HCV-specific CD8$^+$ lytic activity from the peripheral blood of Talbot (CH-561, Fig. 1) and Tardy (CH-445, Fig. 2) against autologous, ^{51}Cr-labelled B cell lines sensitized with the homologous peptide pool 3 but not with an irrelevant peptide pool representing another region of core. This response was detected only at week 7 post-infection, suggesting that high HCV-specific CTL frequencies are present transiently in the peripheral blood during the acute phase of infection. Although the precursor frequency of these cells was not determined, it is noteworthy that only one round of peptide stimulation was required to detect the lytic activity. Virus replication was detected at all time points, and both animals eventually developed a persistent infection. It is not

clear why the frequency of these HCV-specific cells dropped after week 7, or whether a similar transient pattern of peripheral blood CTL activity would be observed in animals that spontaneously resolve infection. Answers to these questions could provide some insight into mechanisms that govern the outcome of HCV infection.

Summary

Several features of human HCV infection are recapitulated in the chimpanzee model. Most importantly, the frequency of persistent infection is high in both species, and virus replication occurs despite evidence of cellular and humoral immune responses. A key difference is that necroinflammatory lesions in chronically infected chimpanzees are almost always mild, whereas in humans the disease spectrum is very wide, ranging from mild to severe hepatitis and end-stage cirrhosis requiring transplantation. Understanding the basis for both the similarities and differences in persistent hepatitis C in the two species will probably be important for the development of effective prevention and therapy of HCV infection.

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